Physician Instructions for Collecting and Handling Clinical Specimens and Data

Study of Patients with Tick Bite-Associated Rash Lesions of Unknown Etiology in the Southern United States

Background

Lyme disease is due to infection with the tick-transmitted spirochete, *Borrelia burgdorferi*. In the United States, the regions with the highest incidences of Lyme disease are the Northeast, Upper Midwest, and northern Pacific Coast. The characteristic annular, macular, erythematous skin lesion of early Lyme disease, erythema migrans (EM), occurs at the site of the infected tick bite, has an incubation period of 3-31 days, and typically expands over time, sometimes to a diameter of $30 cm.

Tick bite-associated EM-like lesions also occur in the southern United States, but the etiology of such lesions is unknown. Some appear to be associated with bites of the Lone Star tick, *Amblyomma americanum*, which is the most common human-biting tick in the region. Studies to date have failed to convincingly implicate *B. burgdorferi* as the cause of the rash. Possible etiologies include a novel tick-transmitted spirochete, called *Borrelia lonestari*, another infectious agent, or some other inflammatory process.

To determine the etiology and epidemiology of tick-associated annular skin lesions in the South, scientists at the Centers for Disease Control and Prevention (CDC) are cooperating with clinicians to collect appropriate clinical material for research purposes. Skin specimens will be tested by polymerase chain reaction (PCR) for *Borrelia* DNA sequences and evaluated by microscopy. Once tick cell culture methods have been established at CDC, a portion of each skin and blood sample will be co-cultured with tick cells in an attempt to isolate *B. lonestari.* Serum samples will not be tested immediately, but will be stored in a confidential manner until serologic tests for antibodies to *B. lonestari* have been developed. Portions of each type of sample will be stored to permit future testing of various etiologic hypotheses, once test methods are available. Future tests on these specimens will be identified to the CDC IRB and approved by the IRB as an amendment to this protocol before they are performed. Research subjects and unaffiliated investigators will not be informed of these test results.

Your cooperation is important to insure that informed patient consent is obtained, appropriate clinical specimens are obtained and properly handled, and that standardized and complete clinical data are collected. Please read carefully the following guidelines and any enclosures or attachments. If you have technical questions, wish to inquire about the availability of supplies for specimen collection, or wish to have copies of pertinent publications in the scientific literature, please contact one of the CDC scientists listed below.

Patient Eligibility

The following criteria should be used to determine if a patient is eligible for enrollment:

1. A person at least three years old with acute onset (within 14 days of visit to a physician's office) of an annular, erythematous, expanding EM-like rash that attains a size of at least 5 cm in diameter, when no alternative explanation for the rash can be found, and with

2. A history of tick bite at the rash site or potential exposure to ticks within 14 days prior to rash onset, and with

3. A willingness to consent to storage of all biologic samples that are donated for future laboratory testing. An amended IRB protocol will be submitted for review of any proposed additional testing. Such tests will not be performed until the amended protocol is approved.

Your participation

If you wish to be a collaborating physician, please read a summary of the ethical principles and guidelines for the protection of human subjects of research known as the *Belmont Report*, which is in this study packet. Please then read and sign the Unaffiliated Investigator Agreement (UIA) indicating that you will protect the rights and welfare of human subjects involved in research under this CDC Institutional Review Board (IRB)-approved protocol. **You must sign and return the UIA or you may not submit specimens.**

Informed Consent for adults and Assent forms for minors

1. Adult patients: PRIOR to collecting specimens, please ask adult patients who are legally able to give consent to read and sign the Adult Consent/ Parental Permission/ Adolescent Assent form.
2. Patients between 15 and 17 years old: A parent or guardian should give permission by signing an Adult Consent/ Parental Permission/ Adolescent Assent form and the minor child should give assent by signing another copy of the Adult Consent/ Parental Permission/ Adolescent Assent form.
3. Patients between 7 and 14 years old: A parent or guardian should give permission by signing an Adult Consent/ Parental Permission/ Adolescent Assent form and the minor child should give assent by signing a Child's Assent form for Minors.
4. Patients between 3 and 7 years old: We will enroll children older than 3 with the permission of a parent or guardian. An Adult Consent/ Parental Permission/ Adolescent Assent form signed by a legally responsible adult is sufficient for enrollment of a child aged 3-7.

It is extremely important that your office return the signed and witnessed form(s) with the clinical specimens. **We cannot accept specimens and enroll patients in the study without signed informed consent and, when appropriate, an assent form for minors.**

Laboratory Submission Form for Data Collection

Please carefully complete the attached *Laboratory Submission Form for Southern Tick-Associated Rash Illness (STARI) Specimens.* Do not include the patient's name on the form. If possible, take a color photograph of the skin lesion, label it, and attach it to the Laboratory Submission form or submit a digital picture by e-mail to bjj1@cdc.gov. [Note: Clinical information that is legible and as complete as possible must accompany all specimens.]

If you wish to request standard serologic tests for Lyme disease, please also submit the enclosed CDC DASH form with the patient's name. Results of standard tests will be sent to you and to your state health department.

Clinical Specimen Collection and Handling

Ideally, the following samples should be collected. A specimen collection kit can be sent to your office prior to the beginning of tick season, or by overnight delivery service at the time a patient presents for care.

1) Two skin biopsy specimens, although one sample is valuable (see submission form).
2) Clotted blood for serum (acute-phase specimen now and a convalescent-phase specimen 3-6 weeks later)
3) Anti-coagulated whole blood

If a patient does not consent to a skin biopsy, it is still important to collect and submit the blood specimens and the clinical data. Please ask the patient or guardian to schedule an appointment 3-6 weeks from the initial visit on the day that the acute-phase blood samples are collected. Collect a convalescent-phase serum sample at the second visit. If the patient misses the second appointment, please call the patient at home to reschedule the serum collection.

Skin biopsies

NOTE: Please exclude persons with hemophilia or other coagulopathies, including patients taking potent anticoagulants such as warfarin. (Patients taking NSAIDs alone need not be excluded.) Also, please exclude immunocompromised patients and persons who are receiving chemotherapy. ***Do not biopsy facial or neck lesions for this study.***

To collect the one, or preferably two, skin biopsy specimens, use a standard sterile 2-mm punch instrument and sterile technique. Ideally, samples should be taken from 4-6 mm inside the outer margin of the annular EM-like skin lesion and within 2.5 cm of each other and to a depth of 3-4 mm. The biopsy sites should be anesthetized (0.5 ml of a 1% solution of lidocaine and epinephrine 1:100,000 at each site) and then disinfected with a tincture of iodine followed by an alcohol swab. Gently twist the punch instrument to cut the skin to a depth of only 3-4 mm. Remove the punch instrument and repeat for the second biopsy. Grasp each skin sample with fine-tipped forceps, pull it gently away from the body, and snip it at its base with iris scissors. If necessary, place the samples on a sterile gauze patch momentarily while attending to hemostasis. Hemostasis is usually achieved by pressure alone; a butterfly bandage or single nonabsorbable suture can be applied if necessary. Place one biopsy specimen into phosphate-buffered saline (tube enclosed) and refrigerate it. Place a second specimen, when available, into Streck tissue fixative (tube enclosed) and store it at room temperature. Both skin samples should be shipped on a gel-type ice pack (see below).

Instruct patients in the proper care of their skin biopsy site. Provide a copy of the wound care instruction sheet to each research subject or guardian.

If only one biopsy specimen is obtained, please place it in PBS transport medium.

Phlebotomy

Disinfect phlebotomy site(s) with a tincture of iodine followed by an alcohol swab. To minimize the risk of hematoma, apply direct pressure to phlebotomy site(s) with the arm straight and elevated for 3-4 minutes.

Clotted blood for serum

Collect a 10-ml acute-phase blood sample in the enclosed standard red/gray-topped serum separator tube and centrifuge it in the standard fashion. Store the sample at approximately 4EC and ship it to CDC on a gel-type ice pack. It is unnecessary to decant the serum from the clot after centrifugation. It is important that a convalescent-phase sample be collected 3-6 weeks later in a similar fashion and shipped on a gel-type ice pack.

If, in your judgment, less than a full 10-ml sample from a child is more appropriate, please collect only this lesser amount.

Uncoagulated whole blood

Collect a 5-ml blood sample in an EDTA-coated (purple-topped) tube in the standard fashion. Store it at approximately 4EC and ship it on a gel-type ice pack.

If, in your judgment, less than a full 5-ml sample from a child is more appropriate, please collect only this lesser amount.

Shipping and Reimbursement

CDC will pay for overnight shipping if you ask for a CDC Federal Express account number from one of the

CDC scientists listed below. **Reimbursement is not possible if you pay for shipping.**

CDC will pay fees for phlebotomy and skin biopsy procedures, if arranged in advance. Skin biopsy expenses should not be charged to participants, since this procedure is not routine for evaluation of rash illness.

Patients will not be paid for their participation.

Ship samples to:

Bacterial Zoonoses Branch
CDC
Foothills Campus (Rampart Road)
Fort Collins, CO 80521-2087
ATTN: Mr. Steve Sviat
(970) 221-6400

CDC Scientist Contacts

Dr. Barbara Johnson (Microbiology and Pathogenesis Laboratory)
Tel (970) 221-6463, E-mail bjohnson@cdc.gov

Dr. Jacob Kool (Epidemiology Activity)
Tel (970) 266-3540, E-Mail jkool@cdc.gov

Dr. Paul Mead (Epidemiology Activity)
Tel (970) 221-6474, E-mail pmead@cdc.gov

Bacterial Zoonoses Branch
Division of Vector-Borne Infectious Diseases
CDC
P.O. Box 2087
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